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INTERNATIONAL PRELIMINARY EXAMINATION REPORT (PCT Article 36 and Rule 70)

Applicant's or agent's file reference P101117WO	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA/416)	
International application No. PCT/GB 03/04296	International filing date (day/month/year) 03.10.2003	Priority date (day/month/year) 07.10.2002
International Patent Classification (IPC) or both national classification and IPC C07K14/47		
Applicant LUDWIG INSTITUTE FOR CANCER RESEARCH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.


2. This REPORT consists of a total of 6 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the opinion
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 19.04.2004	Date of completion of this report 08.10.2004
Name and mailing address of the International preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Young, C Telephone No. +49 89 2399-7877



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EXAMINATION REPORT**

International application No. PCT/GB 03/04296

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, Pages

1-38 as originally filed

Claims, Numbers

1-53 received on 24.09.2004 with letter of 24.09.2004

Drawings, Sheets

1-19 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
☐ the language of publication of the international application (under Rule 48.3(b)).
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
☐ filed together with the international application in computer readable form.
☒ furnished subsequently to this Authority in written form.
☒ furnished subsequently to this Authority in computer readable form.
☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☒ the claims, Nos.: 54
☐ the drawings, sheets:

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5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-26,31-33
	No: Claims	27-30,34-53
Inventive step (IS)	Yes: Claims	
	No: Claims	1-26,31-33
Industrial applicability (IA)	Yes: Claims	1-53
	No: Claims	

2. Citations and explanations

see separate sheet

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Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents/:

- D1: SAMUELS-LEV YARDENA ET AL: "ASPP proteins specifically stimulate the apoptotic function of p53" MOLECULAR CELL, CELL PRESS, CAMBRIDGE, MA, US, vol. 8, no. 4, October 2001 (2001-10), pages 781-794, XP002202189 ISSN: 1097-2765
- D2: NAUMOVSKI L & CLEARY M L: "The p53-binding protein 53BP2 also interacts with Bcl2 and impedes cell cycle progression at G2/M" MOLECULAR BIOLOGY OF THE CELL, BETHESDA, MD, US, vol. 16, no. 7, 1 July 1996 (1996-07-01), pages 3884-3892, XP002095578 ISSN: 1059-1524
- D3: IWABUCHI KUNIYOSHI ET AL: "Stimulation of p53-mediated transcriptional activation by the p53-binding proteins, 53BP1 and 53BP2" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 273, no. 40, 2 October 1998 (1998-10-02), pages 26061-26068, XP002189291 ISSN: 0021-9258

Intermediate document;

- D4: BERGAMASCHI DANIELE ET AL: "iASPP oncoprotein is a key inhibitor of p53 conserved from worm to human." NATURE GENETICS, vol. 33, no. 2, February 2003 (2003-02), pages 162-167, XP001180301 ISSN: 1061-4036 (ISSN print)

Novelty, Article 33 (2) PCT

The priority of the present application with regard GB0223193 appears valid. As such the intermediate document D4 is not considered prejudicial for novelty.

The present invention relates to two inhibitory isoforms of the ASPP family, one human (figure 1a) the other originating from *C. elegans*, see figure 1b. Although related members of inhibitory ASPP (iASPP) exist such as 53 BP2 and Bbp see D1-D3 the

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particular sequences claimed namely a sequences consisting of a fragment from residue 128-224 are not disclosed. As such claim 1 -26 and 31-33 are considered novel.

Claims 27-30 relate to any nucleic acid hybridizing or hybridizing under stringent conditions from the genus *caenorhabditis*. These claims do not delineate clearly from other iASSPs of the art, see D1-D3. The sequences of these disclosures would be expected to fall within the claimed scope. Note the origin of a particular sequence is not considered to bestow features contributing to novelty. Moreover, D1-D3 disclose a peptide comprising that shown in Figure 2b of the application. In short this Authority consider that claims 27-30 are not novel over D1-D3.

Similarly claims 35 to 53 essentially relate to peptides comprising amino acid sequences disclosed in D1 to D3. This authority consider these claims as lacking novelty over said disclosures.

Inventive step, Article 33 (3) PCT

The cited prior art D1-D3 discloses various C terminal fragments of ASPP as iASPP. Any of D1-D3 may represent the closest prior art. Each discloses an iASPP. However, no mention is made of the N-terminal sequences 128-224 recognized above to represent the novel part of the present application.

"The objective problem is defined as the cloning of further iASPP molecules"

No indication is given in the closest prior art or cited art that the N-terminus of ASPP in the region 128-224 would be an inhibitor of apoptosis. This is truly an unexpected finding and is well documented in the later published Nature paper D4.

In principle claims reciting both the "specific" sequence and biological function namely an inhibitor of apoptosis would indeed be inventive. However, at present the claims relate to the sequence of the invention i.e. region 128-224 plus "additions". This does not clearly define the invention (see sequences of D1-D3) nor does it include the surprising biological activity that would warrant recognition of inventive step. The claimed fragments may well not even possess the feature that makes them inventive. The latter problem arises as claim 1 is written in two parts (i) without function or (ii) with function

As the function is considered to be an essential feature inventive step can not be

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recognized at present.

In short claims 1-26 and 31-33 do not meet the requirements of Article 33 (3) PCT.

Claims

1. An isolated nucleic acid molecule which encodes a polypeptide, or sequence variant thereof, wherein said polypeptide is a fragment of the polypeptide sequence represented in Figure 2a or 2b, which fragment is selected from:
 - i) a polypeptide fragment consisting of amino acid residues from about residue 128-224 of the amino acid sequence presented in Figure 2a or 2b; or
 - ii) a polypeptide fragment consisting of amino acid residues from about residue 128-224 of the amino acid sequence presented in Figure 2a or 2b wherein said sequence has been modified by addition, deletion or substitution of at least one amino acid residue characterised in that said polypeptide inhibits the apoptotic activity of p53.
2. A nucleic acid molecule according to Claim 1 wherein said molecule encodes a fragment consisting of amino acid residues from about residue 128-224 of the sequence represented in Figure 2a.
3. A nucleic acid molecule according to Claim 2 wherein said molecule is isolated from a human.
4. A nucleic acid molecule according to Claim 1 or 2 wherein said molecule encodes a fragment consisting of amino acid residues from about residue 128-224 of the sequence represented in Figure 2b.
5. A nucleic acid molecule according to Claim 4 wherein said molecule is isolated from a nematode.
6. A nucleic acid molecule according to Claim 5 wherein said nematode is of the genus *Caenorhabditis* spp.

7. A nucleic acid molecule according to any of Claims 1-6 wherein said nucleic acid molecule is a cDNA.
8. A nucleic acid molecule according to any of Claims 1-6 wherein said nucleic acid molecule is genomic DNA.
9. A polypeptide fragment or sequence variant thereof, encoded by a nucleic acid molecule according to any of Claims 1-8.
10. A vector comprising a nucleic acid according to any of Claims 1-8.
11. A vector according to Claim 10 wherein said vector is an expression vector.
12. A cell transformed or transfected with a nucleic acid molecule according to any of Claims 1-8 or a vector according to Claim 10 or 11.
13. A nucleic acid according to any of Claims 1-8 for use as a pharmaceutical.
14. A polypeptide according to Claim 9 for use as a pharmaceutical.
15. A nucleic acid or polypeptide according to Claim 13 or 14 further comprises a diluent, carrier or excipient.
16. A transgenic non-human animal comprising a nucleic acid molecule according to any of Claims 1-11.
17. The use of the polypeptide, or fragment thereof, according to Claim 9 in a screening method for the identification of agents which inhibit the binding of said polypeptide to p53.
18. A screening method to identify agents which inhibit the binding of a polypeptide, or fragment thereof, to p53 comprising:
 - i) forming a preparation comprising
 - a) a polypeptide according to Claim 9; and

- b) a p53 polypeptide, or a fragment thereof consisting of the binding site(s) for the polypeptide in (a);
- ii) providing at least one agent to be tested; and
- iii) determining the activity of the agent with respect to the binding of the polypeptide in (a) to the polypeptide in (b).
19. A method according to Claim 18 wherein said agent is a polypeptide.
20. A method according to Claim 18 wherein said polypeptide is a peptide.
21. A method according to Claim 19 wherein said polypeptide is an antibody or binding part thereof.
22. A method according to Claim 21 wherein said antibody is a monoclonal antibody.
23. A method according to Claim 21 or 22 wherein said fragment is a Fab fragment.
24. A method according to Claim 23 wherein said Fab fragment is selected from the group consisting of: $F(ab')_2$, Fab, Fv and Fd fragments; or CDR3 regions.
25. A method according to any of Claims 22-24 wherein said antibody is a humanised.
26. A method according to any of Claims 22-24 wherein said antibody is a chimeric antibody.
27. An isolated nucleic acid molecule wherein said molecule is isolated from a nematode worm which nucleic acid molecule hybridises a nucleic acid sequence as represented by Fig 2b, wherein said nucleic acid molecule encodes an inhibitor of p53 and inhibits the apoptotic activity of p53.
28. A nucleic acid molecule according to Claim 27 wherein said molecule

hybridises under stringent hybridisation conditions.

29. A nucleic acid molecule according to Claim 27 or 28 wherein said nematode worm is of the genus *Caenorhabditis* spp.

30. An isolated polypeptide comprising the amino acid as represented in Figure 2b or a variant polypeptide which polypeptide is modified by addition, deletion or substitution of at least one amino acid residue and is an inhibitor of p53.

31. A method of treatment of an animal comprising administering an effective amount of a polypeptide according to Claim 9 wherein said effective amount induces the apoptotic activity of p53.

32. A method of treatment of an animal comprising administering an effective amount of a nucleic acid molecule according to any of Claims 1-8 or a vector according to Claim 10 or 11 wherein said effective amount induces the apoptotic activity of p53.

33. A method according to Claim 31 or 32 wherein said treatment is of cancer.

34. A peptide comprising an amino acid sequence selected from the group consisting of: DGPEETD; GPEETD; TTLSDG; AEEFGDE; or PRNYFG.

35. A peptide according to Claim 34 wherein the length of said peptide is at least 6 amino acid residues.

36. A peptide according to Claim 34 wherein the length of said peptide is selected from the group consisting of: is at least 7 amino acid residues; 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid residues.

37. A peptide according to Claim 34 wherein the length of said peptide is at least 20 amino acid residues; 30; 40; 50; 60; 70; 80; 90; or 100 amino acid residues.

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38. A peptide according to Claim 34 consisting of an amino acid sequence selected from the group consisting of: DGPEETD; GPEETD; TTLSDG; AEEFGDE; or PRNYFG.

39. A peptide according to any of Claims 34-38 wherein said peptide further comprises a plurality of arginine residues.

40. A peptide according to Claim 39 wherein said plurality of arginine residues is at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 arginine residues in length.

41. A peptide selected from the group consisting of: DGPEETD; GPEETD; TTLSDG; AEEFGDE; or PRNYFG for use as a pharmaceutical.

42. A pharmaceutical composition comprising a peptide selected from the group consisting of: DGPEETD; GPEETD; TTLSDG; AEEFGDE; or PRNYFG.

43. A pharmaceutical composition according to Claim 42 wherein said composition further includes a carrier, diluent or excipient.

44. A pharmaceutical composition comprising at least one peptide according any of Claims 34-40 and at least one anti-cancer agent.

45. A pharmaceutical composition according to Claim 44 wherein said anticancer agent is selected from the group consisting of: cisplatin; carboplatin; cyclophosphamide; melphalan; carmustine; methotrexate; 5-fluorouracil; cytarabine; mercaptopurine; daunorubicin; doxorubicin; epirubicin; vinblastine; vincristine; dactinomycin; mitomycin C; taxol; L-asparaginase; G-CSF; etoposide; colchicine; derferoxamine mesylate; and camptothecin.

46. A pharmaceutical composition according to Claim 45 wherein said agent is cisplatin.

47. A pharmaceutical composition according to Claim 45 wherein said agent is doxorubicin.

48. A complex comprising a peptide according to any of Claims 34-40 and an antibody, or binding part thereof.

49. A complex according to Claim 48 wherein said antibody or binding part is a cell specific antibody.

50. A complex according to Claim 48 or 49 wherein said antibody is a cancer cell specific antibody.

51. A method of treatment of an animal, preferably a human, wherein said animal would benefit from the induction of apoptosis comprising administering an effective amount of a peptide according to any of Claims 34-40.

52. A method of treatment of an animal, preferably a human, wherein said animal would benefit from the induction of apoptosis comprising administering an effective amount of a composition according to any of Claims 42-47 or a complex according to any of Claims 48-50.

53. A method according to Claim 51 or 52 wherein said treatment is cancer treatment.

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AMENDED SHEET